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Risk of Transmission of Herpesviruses through Cord Blood Transplantation

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ABSTRACT

Cord blood (CB) progenitor cells are increasingly used for transplantation in children because of the lower risk of graft-versus-host disease compared with unrelated bone marrow and comparable rates of disease-free survival. There is concern that CB might carry a higher risk of opportunistic infections. Human herpesviruses (HHV) are common pathogens in transplant recipients. CB donors are routinely tested for the presence of anti-cytomegalovirus (CMV) immunoglobulin M to reduce the risk of collecting CMV-infected CB. To assess the incidence of β and γ HHV infection of CB collected under standard procedures, we tested 362 CB samples for the presence of CMV; HHV-6, -7, and -8; and Epstein-Barr virus DNA by polymerase chain reaction. HHV-6 DNA was found in 2 samples, yielding an incidence of 0.55% (95% confidence interval, 0.1%-2%). None of the other viral DNAs was found, resulting in a 95% confidence interval of 0% to 1% for the incidence of CMV, Epstein-Barr virus, HHV-7, and HHV-8. Because the seroprevalence of HHV-8 among the CB donors in this study was only 4%, these findings cannot be extended to HHV-8–endemic areas. Our data show that screening prospective CB donors with anti-CMV immunoglobulin M practically eliminates the risk of CB CMV transmission, but HHV-6 warrants CB testing by polymerase chain reaction.

KEY WORDS

Cord blood • Human herpesvirus–6 • Cytomegalovirus • Epstein-Barr virus

INTRODUCTION

Umbilical cord blood (CB)–derived progenitor cells are increasingly used to restore hematopoiesis in patients who require bone marrow transplantation. Since the first CB transplantation was performed by Gluckman et al. in 1988 [1], more than 3000 patients worldwide have received related or unrelated CB transplants for a variety of malignant and nonmalignant diseases [2,3]. The event-free survival rates reported thus far are comparable to results achieved after unrelated allogeneic marrow transplantation. Moreover, there are many reports of less graft-versus-host disease than with marrow transplantations [4,5], particularly in pediatric patients. CB donors are typically screened for the most common blood-borne pathogens and disqualified if there is serologic evidence of infection with *Treponema pallidum*, human immunodeficiency virus (HIV)–1 and -2, human T lymphotropic viruses 1 and 2, hepatitis B (HBV) and C viruses, or recently acquired cytomegalovirus (CMV).

In addition to CMV, other β and γ human herpesviruses (HHV) have the potential of establishing infection of circulating mononuclear cells and contaminating the CB infusion. Severe disease consequent to hematopoietic cell-associated transmission of HHV-6 [6], HHV-8 [7], and Epstein-Barr virus (EBV) [8,9] has been recorded. Although congenital EBV infection is extremely rare [10], in utero transmission of HHV-6 and -8 has been reported at rates varying from 1% to 1.6% [11,12] and 0.4% [13], respectively. A comprehensive literature review failed to reveal any published studies of congenital HHV-7 infection. In this study, we examined the prevalence of β and γ HHV DNA in banked CB samples.

MATERIALS AND METHODS

CB Samples

Frozen CB was obtained from the CB bank at the University of Colorado Health Sciences Center. Potential donors, identified and enrolled according to

Table 1. *Sequences of Primers and Probes Used for HHV β and γ Multiplex PCR*

Virus	Primer Pair 5′ → 3′	Probe 5′ → 3′
CMV	GGC AGC TAT CGT GAC TGG GA GAT CCG ACC CAT TGT CTA AG	ATT CGT GGT CGT GGC CAA CTG GTG CTG CCG GTC GCG CTT A
EBV	GTC AAC CAA CAA GGA CAC AT CAC CAC CTT GTT TTG ACG GG	CCG CGG GAG CTA GGG GCA GG
HHV-6	AAG CTT GCA CAA TGC CAA AAA ACA G CTC GAG TAT GCC GAG ACC CCT AAT C	AAC TGT CTG ACT GGC AAA AAC TTT T
HHV-7	TAT CCC AGC TGT TTT CAT ATA GTA AC GCC TTG CGG TAG CAC TAG ATT TTT TG	AGA ATT CTG TAC CCAT GGG CAC ATT TGT AC
HHV-8	TCC GTG TTG TGT ACG TGG AG AGC CGA AAG GAT TCC ACC AT	CCA TGG TCG TGC CGC AGC A

the local investigational review board–approved protocol, were tested for the presence of anti–HIV-1 and -2, HIVP24, human T lymphotropic virus 1 and 2, hepatitis C virus, and HBV immunoglobulin (Ig)G antibodies; HBV surface antigen; anti-CMV IgM antibodies; and rapid plasma reagin. Of these, CMV IgM screening is specific to our blood bank and may not be performed at other centers. CB were collected from the donors with negative results for all these tests. In addition, aliquots of whole and volume-reduced CB, as well as serum from the CB and the mother, were frozen for subsequent testing and screening out infected units. When a candidate CB recipient was identified, one of the frozen aliquots was further tested for CMV, parvovirus B 19, and HIV-1 by polymerase chain reaction (PCR).

HHV DNA Measurements

Multiplex PCR was performed by using the primers and probes previously validated in our laboratory [14-17] (Table 1). DNA was extracted from 200 μL of cryopreserved whole blood with QIAamp DNA purification columns (Qiagen, Valencia, CA). The multiplex PCR was performed in 50 μL of final volume containing 20 μL of extracted DNA, 1.25 U of *Pfu*I (Stratagene, La Jolla, CA), 200 μmol/L of each of 4 deoxynucleoside triphosphates, and 0.5 μmol/L of each of the biotinylated primers in *Pfu*I buffer (Stratagene). The samples were amplified for 45 cycles. The amplified DNA was allowed to bind to capture probes (0.5 μg/mL in 1 mol/L ammonium acetate) previously immobilized onto 96-well enzyme immunoassay plates. Bound amplicon was detected with streptavidin/horseradish peroxidase (R&D Systems, Minneapolis, MN) and tetranitrotetrazolium blue colorimetric substrate (R&D Systems). The optical density (OD) was measured at 450 nm with an enzyme immunoassay reader. A sample with an OD ≥0.6 was considered positive for the presence of the DNA of interest. This method has a limit of detection (LOD) of ≥2 copies per reaction tube of each of the targeted viral DNAs. This corresponds to a LOD of ≥1 copy per 10^3 CB mononuclear cells. If infected cells contained only 1 copy of viral DNA, then the LOD for infected cells would be ≥0.1% of the CB mononuclear cells. However, because infected cells typically contain multiple copies of viral DNA, a more accurate LOD is approximately 0.01% of infected cells. Positive and negative controls were included in each run. The runs were considered valid if the absorbance of the negative control was <0.3 and that of the positive control was >1.0. Multiplex PCR–positive results were confirmed by a single PCR consisting of amplification and Southern blot detection as previously described [14-17].

HHV Serology

The IgG antibodies anti-CMV, anti-EBV, anti-HHV-6, and anti-HHV-8 were detected with commercially available enzyme-linked immunosorbent assay kits according to the manufacturers' instructions (CMV, Diamedix, Miami, FL; EBV, Sigma, St. Louis, MO; HHV-6 and -8, Advanced Biotechnologies Inc., Columbia, MD). Anti-HHV-7 IgG antibodies were measured with HHV-7 purified viral antigen (Advanced Biotechnologies Inc.), anti-human IgG/horseradish peroxidase conjugate (Sigma), and tetranitrotetrazolium blue colorimetric substrate (R&D Systems). The positive cutoff value was calculated as the mean ± 3 SD of the 3 negative controls included in each run. This method was validated against a commercially available HHV-7 indirect immunofluorescence kit (Advanced Biotechnologies Inc.). Runs were considered valid if the negative and positive controls performed within the expected range, ie, negative controls, OD ≤0.2; low positive controls, OD 0.5 to 1; and high positive controls, OD ≥1.5.

Statistical Methods

Rates of infection and 95% confidence intervals (CI) were calculated on the basis of the binomial distribution.

RESULTS

The study was conducted on 362 randomly selected CB aliquots. IgG antibody testing of the maternal and/or cord sera revealed that 187 mothers

Table 2. *Incidence of β and γ Human Herpesvirus Infections in 362 Cord Blood Samples*

Virus	Ab pos	PCR pos	% Rate of Infection (95% Confidence Interval)	
			Seropositive	Overall
HHV-6	297	2	0.67 (0.08-2.41)	0.55 (0.07-1.98)
HHV-7	335	0	0 (0-1.1)	0 (0-1.01)
CMV	187	0	0 (0-1.95)	0 (0-1.01)
EBV	300	0	0 (0-1.22)	0 (0-1.01)
HHV-8	16	0	0 (0-20.59)	0 (0-1.01)

Ab indicates antibodies; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HHV, human herpesvirus; PCR, polymerase chain reaction; pos, positive.

were CMV seropositive, 300 were EBV seropositive, 297 were HHV-6 seropositive, 335 were HHV-7 seropositive, and 16 were HHV-8 seropositive (Table 2). Two CB samples contained HHV-6 DNA. No other HHV DNA was found. The incidence of HHV-6 infection of CB cells was 0.67% (95% CI, 0.08%-2.4%) among samples obtained from seropositive mothers. The overall incidence of HHV-6 infection of CB was 0.55% (95% CI, 0.07%-1.98%). The incidences of other HHV infections of CB were null, with overall 95% CIs of 0% to 1%. However, the upper limit of the 95% CI adjusted for seropositivity varied from 1.1% for HHV-7 to 20.6% for HHV-8. The latter reflected the low HHV-8 seroprevalence in our CB donor population.

DISCUSSION

This study showed that HHV-6 infection of CB occurred in 0.55% of banked samples obtained from HHV-6–seropositive mothers (95% CI, 0.1%-2%). The presence of HHV-6 DNA in the specimen that generates the graft indicates that there is a risk of transmission of the virus to the CB recipient. The risk of CB-mediated HHV-6 transmission is also supported by previous studies that showed higher HHV-6 viral loads in CB recipients compared with other types of stem cell transplants and found evidence of primary HHV-6 infection after CB transplantation [18]. HHV-6 infection in allogeneic CB recipients is associated with clinical manifestations ranging from fever and rash to encephalitis, pneumonitis, and bone marrow suppression [6,19-23]. CB transplantation is more likely to be used in childhood and to introduce the virus to the host for the first time. It has also been demonstrated that CB recipients use their prior immunologic memory to reconstitute immune responses [24]. When faced with a primary infection after transplantation, the host will not be able to use immunologic memory to develop a protective response against HHV-6. Whether this might impair the ability of the host to mount a competent immune response remains to be determined. A conservative approach at this point would be to avoid using HHV-6 PCR-positive CB samples whenever possible.

The incidence of CMV infection was 0% (95% CI, 0%-1%) in the CB units obtained from CMV IgM-negative pregnancies, even though the overall incidence of congenital CMV infection in the United States varies between 1% and 2% [25]. This is consistent with previous reports that the presence of CMV IgM in the serum of pregnant women identifies most cases at risk of in utero CMV transmission [26]. Furthermore, CMV PCR of the CB or infant's blood is invariably positive in cases of congenital CMV infection [27]. Taken together, this indicates that excluding CB donations from CMV IgM–seropositive mothers is an effective measure to prevent CB-associated CMV transmission and could potentially be embraced by other centers, which currently do not routinely test pregnant volunteers for CMV IgM. Our CB bank routinely screens all CB by CMV PCR before clinical use. Since October 1996, out of 312 CB samples screened in this fashion, only 1 had a positive result. Adding these numbers to the results of the 362 CB samples tested in this study shows a composite risk of CB infection with CMV of 0.15% (95% CI, 0.04%-0.82%), which further underscores the effectiveness of the CMV IgM screening procedure.

There were no cases of EBV, HHV-7, or HHV-8 infections of the CB units. EBV de novo infection leading to posttransplantation lymphoproliferative disorder has been described after bone marrow transplantation [8], but not after CB transplantation. This is consistent with the absence of EBV DNA in CB units and with the extremely low incidence of congenital EBV infection [28]. HHV-7 has been less studied in the setting of CB. Although HHV-7 shares many epidemiologic and clinical characteristics with HHV-6 and CMV, HHV-7 reactivations seem to be less frequent than those of other β HHVs, both in pregnancy [29] and in CB recipients [30]. Furthermore, HHV-7 has not yet been associated with symptomatic disease in CB recipients, thus indicating that screening CB for HHV-7 infection might not be necessary. HHV-8 has been reported as the cause of fatal bone marrow failure in an autologous stem cell recipient [7] and therefore deserves special attention in other transplantation settings. In utero transmission of HHV-8 is less than 1% in endemic areas [13]. This, coupled with the low seroprevalence of HHV-8 in our environment, explains the absence of HHV-8 infection in our CB samples.

Patients undergoing CB transplantation benefit from a low incidence of graft-versus-host disease but might experience more frequent infectious complications. Among β and γ HHVs, HHV-6 and CMV require CB screening, but HHV-7 and EBV do not. HHV-8 infection of CB seems to be a minor problem in areas of low seroprevalence, but it needs to be

further studied in highly endemic areas. Other potential CB infections, including parvovirus B19, rubella, and toxoplasmosis—as well as Chagas disease and malaria in endemic areas—also need to be studied.

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REFERENCES

1. Gluckman E, Devergie A, Bourdeau-Esperou H, et al. Transplantation of umbilical cord blood in Fanconi's anemia. *Nouv Rev Fr Hematol.* 1990;32:423-425.
2. Laughlin MJ, Barker J, Bambach B, et al. Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. *N Engl J Med.* 2001;344:1815-1822.
3. Rubinstein P, Carrier C, Scaradavou A, et al. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *N Engl J Med.* 1998;339:1565-1577.
4. Rocha V, Wagner Jr, JE Sobocinski KA, et al. Graft-versus-host disease in children who have received a cord-blood or bone marrow transplant from an HLA-identical sibling. Eurocord and International Bone Marrow Transplant Registry Working Committee on Alternative Donor and Stem Cell Sources. *N Engl J Med.* 2000;342:1846-1854.
5. Barker JN, Davies SM, DeFor T, Ramsay NK, Weisdorf DJ, Wagner JE. Survival after transplantation of unrelated donor umbilical cord blood is comparable to that of human leukocyte antigen-matched unrelated donor bone marrow: results of a matched-pair analysis. *Blood.* 2001;97:2957-2961.
6. Drobyski WR, Dunne WM, Burd EM, et al. Human herpesvirus-6 (HHV-6) infection in allogeneic bone marrow transplant recipients: evidence of a marrow-suppressive role for HHV-6 in vivo. *J Infect Dis.* 1993;167:735-739.
7. Cuzzola M, Irrera G, Iacopino O, et al. Bone marrow failure associated with herpesvirus 8 infection in a patient undergoing autologous peripheral blood stem cell transplantation. *Clin Infect Dis.* 2003;37:e102-e106.
8. Shearer WT, Ritz J, Finegold MJ, et al. Epstein-Barr virus-associated B-cell proliferations of diverse clonal origins after bone marrow transplantation in a 12-year-old patient with severe combined immunodeficiency. *N Engl J Med.* 1985;312:1151-1159.
9. Curtis RE, Travis LB, Rowlings PA, et al. Risk of lymphoproliferative disorders after bone marrow transplantation: a multi-institutional study. *Blood.* 1999;94:2208-2216.
10. Joncas JH, Alfieri C, Leyritz-Wills M, et al. Simultaneous congenital infection with Epstein-Barr virus and cytomegalovirus. *N Engl J Med.* 1981;304:1399-1403.
11. Adams O, Krempe C, Kogler G, Wernet P, Scheid A. Congenital infections with human herpesvirus 6. *J Infect Dis.* 1998;178:544-546.
12. Dahl H, Fjaertoft G, Norsted T, Wang FZ, Mousavi-Jazi M, Linde A. Reactivation of human herpesvirus 6 during pregnancy. *J Infect Dis.* 1999;180:2035-2038.
13. Brayfield BP, Phiri S, Kankasa C, et al. Postnatal human herpesvirus 8 and human immunodeficiency virus type 1 infection in mothers and infants from Zambia. *J Infect Dis.* 2003;187:559-568.
14. Ansari A, Li S, Abzug MJ, Weinberg A. Human herpes virus-6 and 7 are uncommon causes of central nervous system infection in children. *Emerg Infect Dis.* 2004;10:1450-1454.
15. Campbell TB, Borok M, White IE, et al. Relationship of Kaposi sarcoma (KS)-associated herpesvirus viremia and KS disease in Zimbabwe. *Clin Infect Dis.* 2003;36:1144-1151.
16. Weinberg A, Spiers D, Cai GY, Long CM, Sun R, Tevere V. Evaluation of a commercial PCR kit for diagnosis of cytomegalovirus infection of the central nervous system. *J Clin Microbiol.* 1998;36:3382-3384.
17. Weinberg A, Li S, Palmer M, Tyler KL. Quantitative CSF PCR in Epstein-Barr virus infections of the central nervous system. *Ann Neurol.* 2002;52:543-548.
18. Sashihara J, Tanaka-Taya K, Tanaka S, et al. High incidence of human herpesvirus 6 infection with a high viral load in cord blood stem cell transplant recipients. *Blood.* 2002;100:2005-2011.
19. Cone RW, Huang ML, Corey L, Zeh J, Ashley R, Bowden R. Human herpesvirus 6 infections after bone marrow transplantation: clinical and virologic manifestations. *J Infect Dis.* 1999;179:311-318.
20. Imbert-Marcille BM, Tang XW, Lepelletier D, et al. Human herpesvirus 6 infection after autologous or allogeneic stem cell transplantation: a single-center prospective longitudinal study of 92 patients. *Clin Infect Dis.* 2000;31:881-886.
21. Wang FZ, Linde A, Hagglund H, Testa M, Locasciulli A, Ljungman P. Human herpesvirus 6 DNA in cerebrospinal fluid specimens from allogeneic bone marrow transplant patients: does it have clinical significance? *Clin Infect Dis.* 1999;28:562-568.
22. Yoshikawa T, Asano Y, Ihira M, et al. Human herpesvirus 6 viremia in bone marrow transplant recipients: clinical features and risk factors. *J Infect Dis.* 2002;185:847-853.
23. Zerr DM, Gooley TA, Yeung L, et al. Human herpesvirus 6 reactivation and encephalitis in allogeneic bone marrow transplant recipients. *Clin Infect Dis.* 2001;33:763-771.
24. Montagna D, Locatelli F, Moretta A, et al. T lymphocytes of recipient origin may contribute to the recovery of specific immune response toward viruses and fungi in children undergoing cord blood transplantation. *Blood.* 2004;103:4322-4329.
25. Fowler KB, Stagno S, Pass RF. Maternal immunity and prevention of congenital cytomegalovirus infection. *JAMA.* 2003;289:1008-1011.
26. Lazzarotto T, Varani S, Guerra B, Nicolosi A, Lanari M, Landini MP. Prenatal indicators of congenital cytomegalovirus infection. *J Pediatr.* 2000;137:90-95.
27. Halwachs-Baumann G, Genser B, Pailer S, et al. Human cytomegalovirus load in various body fluids of congenitally infected newborns. *J Clin Virol* 2002;25(suppl 3):S81-S87.
28. Straus SE, Cohen JI, Tosato G, Meier J. NIH conference. Epstein-Barr virus infections: biology, pathogenesis, and management. *Ann Intern Med.* 1993;118:45-58.
29. Okuno T, Oishi H, Hayashi K, Nonogaki M, Tanaka K, Yamanishi K. Human herpesviruses 6 and 7 in cervixes of pregnant women. *J Clin Microbiol.* 1995;33:1968-1970.
30. Boutolleau D, Fernandez C, Andre E, et al. Human herpesvirus (HHV)-6 and HHV-7: two closely related viruses with different infection profiles in stem cell transplantation recipients. *J Infect Dis.* 2003;187:179-186.